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Adoptive transfer of allergen-specific CD4$^+$ T cells induces airway inflammation and hyperresponsiveness in Brown–Norway rats

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SUMMARY

Following allergen exposure, sensitized Brown–Norway rats develop airway hyperresponsiveness (AHR) and eosinophilic inflammation together with an increase in activated T cells (CD25$^+$) in the airways. We tested the hypothesis that CD4$^+$ T cells are involved directly in the acquisition of AHR. Spleen T cells from animals that were injected intraperitoneally on three consecutive days with ovalbumin/Al(OH)$_3$, showed a dose-dependent proliferative response *in vitro* to ovalbumin, but not to bovine serum albumin, as measured by [^{3}H]thymidine uptake. For total T-cell transfer, spleen cells obtained from donor rats 4 days after sensitization were depleted of adherent cells by a nylon wool column separation. CD4$^+$ and CD8$^+$ T cells were purified by immunomagnetic beads cell separation. Recipient naive rats were injected intravenously with 50×10^6 total T cells, 20×10^6 and 5×10^6 CD4$^+$ cells, and 5×10^6 CD8$^+$ cells, and were exposed to ovalbumin aerosol 24 hr afterwards. After a further 24 hr, airway responsiveness to acetylcholine (ACh) was measured and provocative concentration (PC) values (PC_{100}, PC_{200} and PC_{300}) (the ACh concentration needed to achieve 100, 200 and 300% increase in lung resistance above baseline) were calculated. Airway responsiveness was significantly increased in recipients of sensitized total T cells compared with recipients of cells from saline-injected donor rats ($P<0{\cdot}05$). There were significantly increased eosinophil major basic protein (MBP)$^+$ cell counts/mm^2 in airway submucosal tissue in the hyperreactive rats and a significant correlation was found between the number of MBP$^+$ cells and PC_{100} ($r = 0{\cdot}75$; $P<0{\cdot}03$) in recipients of sensitized total T cells. Purified CD4$^+$ T cells from sensitized donors induced AHR in naive recipients ($P<0{\cdot}05$), while sensitized CD8$^+$ and naive CD4$^+$ cells failed to do so. Our data indicate that T cells may induce AHR through an eosinophilic airway inflammation and that CD4$^+$ T cells may have a direct effect in this process in Brown–Norway rats.

INTRODUCTION

Asthma is characterized by reversible airflow obstruction, eosinophil-rich airway inflammation and airway hyperresponsiveness (AHR). Studies have suggested that airway inflammation is causally related to AHR and that these changes may be regulated by products from activated T cells.[1–12] Using the Brown–Norway (BN) rat model we have previously performed a series of studies investigating the role of T cells in the pathogenesis of allergic AHR. In these experiments AHR was associated with ovalbumin-specific IgE production, significant increases in eosinophil, neutrophil and lymphocyte counts, and an elevation of CD25$^+$ cells in the bronchoalveolar lavage fluid and in the airway mucosal tissue in rats actively sensitized to and challenged with ovalbumin.[13–16] Between the numbers of CD25$^+$ cells, eosinophils and the extent of AHR there were significant correlations, suggesting a causal relationship. In addition, lung tissue from rats sensitized and exposed to ovalbumin expressed mRNA for interelukin-4 (IL-4) and IL-5 with a reduction in interferon-γ (IFN-γ).[16] These studies demonstrated that, along with AHR, increased serum IgE and airway eosinophilia, the BN rat model shares cardinal features with human atopic asthma, including T-cell activation and an involvement of T-helper-2 (Th2)-type cytokines. Direct evidence, however, is needed to support the regulatory role for T cells in the development of allergic AHR.

Adoptive transfer of sensitized T lymphocytes has been employed previously to determine the role of these cells in a number of immunological conditions. Transfer of CD4$^+$ T cells from immunized animals or specific cell lines has been used to

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Abbreviations: ACh, acetylcholine; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; BSA,bovine serum albumin; c.p.m., count per minute; MBP, major basic protein; OVA, ovalbumin; PC, provocative concentration.

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achieve protective immunity to intestinal nematodes[17] and to induce hypersensitivity pneumonitis[18] or local tissue inflammation,[19] while injection of $CD8^+$ T cells inhibits IgE production after allergic sensitization.[20,21] In a mouse model of delayed-type hypersensitivity, increased contractile responses of the trachea *in vitro* were transferred by T cells.[22–25] Models for transferring T cells have been established recently in the rat[26] and were used to investigate whether T cells are able to induce allergic airway inflammation and functional changes independently of IgE-mediated mechanisms.

The aim of the present experiments was to determine whether adoptively transferred T-cell subsets from allergen-sensitized donors to naive recipients can induce increases in airway responsiveness to acetylcholine following a single exposure to allergen. We also wanted to clarify whether eosinophils play an effector role in the induced changes and, further, whether $CD4^+$, $CD8^+$ or both subclasses of T cells are capable of transferring AHR. These data would provide direct evidence for a regulatory role for T cells in the induction of allergic AHR.

MATERIALS AND METHODS

Animals, sensitization procedures and allergen exposure

Virus-free inbred male Brown–Norway rats (Harlan Olac Ltd, Bicester, UK) (180–230 g) were kept in a special caging system (Maximizer, Theseus Caging Systems Inc., Hazleton, PA) (Table 1).

Sensitized donor rats were injected with ovalbumin (OVA) 1 mg/ml and $Al(OH_3)$ 100 mg/ml in 0·9% NaCl intraperitoneally (i.p.) for 3 consecutive days (days 1–3). Naive donor rats received 0·9% NaCl i.p. without OVA. Four days later (day 7) the spleen was removed under terminal anaesthesia.

A group of naive rats ($n=8$) that received intravenous (i.v.) saline injection, was used to control for baseline values. Recipient rats of total T cells (5×10^7) consisted of two groups receiving either naive ($n=11$) or sensitized cells ($n=18$). Two groups of rats received sensitized (2×10^7 or 5×10^6) and one further group of rats received naive (2×10^7) $CD4^+$ cells ($n=8$ each). Another two groups of rats ($n=8$ each) were injected with either sensitized or naive $CD8^+$ cells (5×10^6). The donor:recipient ratio was 1:1. Aerosol exposure of recipient animals was accomplished as described previously.[13–16] Rats were exposed to 1% (w/v) OVA for 15 min.

Sequence of procedures

In vitro proliferation assays of spleen mononuclear cells from rats were performed to establish the optimal sensitization time–course, and to check effectiveness of sensitization with $OVA/Al(OH)_3$.

Total splenic T-cell populations purified by nylon wool column separation were transferred from sensitized to naive rats (donor:recipient ratio, 1:1). After allergen challenge, airway responsiveness, bronchioalveolar lavage (BAL) cell count and tissue eosinophilia were studied.

T cells were separated into $CD4^+$ and $CD8^+$ subsets and transferred as above (donor:recipient ratio, 2:2).

Table 1. Treatment and baseline resistance of recipient rats

Number of rats (n)	Treatment (i.v. injection)	Baseline resistance (cmH_2O/ml/second)
8	Saline	0·32±0·01
12	Naive T cells (5×10^7)	0·35±0·03
18	Sensitized T cells (5×10^7)	0·42±0·04
8	Naive $CD4^+$ cells (2×10^7)	0·40±0·03
8	Sensitized $CD4^+$ cells (5×10^6)	0·37±0·03
8	Sensitized $CD4^+$ cells (2×10^7)	0·45±0·06
8	Naive $CD8^+$ cells (2×10^7)	0·32±0·03
7	Sensitized $CD8^+$ cells (2×10^7)	0·49±0·06

Enrichment and purification of T cells and adoptive transfer

Spleen cells were purified by density gradient centrifugation (Ficoll–Paque; Pharmacia, Uppsala, Sweden), and resuspended in tissue culture medium (RPMI-1640; Gibco, Paisley, UK). The preparations were depleted of B cells by nylon wool column separation. Viability of the harvested cells was generally greater than 95%. The purity of cells was determined by fluorescence-activated cell sorter (FACS) analysis (Coulter Epics Elite Coulter Electronics Ltd, Luton, UK) using anti-CD2 (AMS Biotechnology Ltd, Whitney, UK).

Purification of $CD4^+$ and $CD8^+$ T cells was carried out by positive selection using anti-mouse IgG Dynabeads (Dynal, Wirral, UK) coated overnight with OX8 (anti-CD8) or w3/25 (anti-CD4). The beads were washed and added ($4·8\times10^7$) to spleen cells ($1·5\times10^7$) and incubated at 4° on a rolling mixer for 45 min. The attached $CD4^+$ or $CD8^+$ cells were collected by using a magnetic particle concentrator (Dynal), and washed in culture medium. Aliquots of the $CD8^+$ or $CD4^+$ T cells were retained and allowed to detach from the magnetic beads in RPMI/10% fetal calf serum (FCS) overnight. The purity of $CD8^+$ and $CD4^+$ T cells was consistently greater than 95%, as assessed by staining with anti-CD3/anti-CD4 and anti-CD8 antibodies and assayed by FACS scan (Coulter Epics Elite).

Purified cells were resuspended in sterile saline in 1-ml volume and injected promptly into the caudal vein of recipient rats, which were lightly anaesthetized (Midazolam and Hypnorm, Janssen Pharmaceuticals, Oxford, UK; 100 μg/100 g i.p.).

Culture of mononuclear cells

The proliferative response of T cells was determined in the presence of OVA and bovine serum albumin (BSA) as control, by uptake of [^{3}H]methylthymidine. Purified spleen cells were cultured in triplicate in sterile 96-well round-bottomed culture plates for 96 hr, or as otherwise stated. For a positive control, we used phytohaemagglutinin (PHA)-induced proliferation (5 μg/ml) that resulted in an average of 20 000 c.p.m. after 48 hr of culture (data not shown). Results are expressed as a proliferation index:

$$\frac{(\text{c.p.m.1}-\text{c.p.m.M})}{\text{c.p.m.M}}$$

where c.p.m.1 = count per minute in the stimulated sample, and c.p.m.M = count per minute in the medium control.

Measurement of bronchial responsiveness

Twenty-four hours after OVA challenge, airway responsiveness to acetylcholine (ACh) was measured in anaesthetized, tracheostomized and ventilated rats as described in our previous studies.[13–16] Changes in lung resistance (R_L) and provocative concentraions PC_{100}, PC_{200} and PC_{300} were calculated from concentration–response curves of individual animals.

BAL and cell counting

Lungs were lavaged as described previously.[13–16] Total and differential cell counts were determined in cytospin preparations stained with May–Grünwald. Cells were identified as macrophages, eosinophils, neutrophils and lymphocytes by standard morphology, and 500 cells were counted for this purpose under ×400 magnification.

Collection of airway tissue and immunocytochemistry

Immunohistochemical staining of cryostat sections (6 μm) was performed as described previously.[15] Slides were read in a blind fashion and in coded random order by two observers using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan). Counts were expressed as positive cells per mm^2 of cross-sectional subepithelial surface. BMK-13, which is an anti-human myelin basic protein (MBP) antibody,[27] has been used for the detection of eosinophils. In a previous study, BMK-13 was confirmed to bind to rat eosinophils, by abolishing its anti-MBP activity following incubation with purified eosinophil granules prior to use in immunocytochemistry.[28]

Data analysis

Results are expressed as mean ± SEM. After performing the Kruskal–Wallis test, and finding significant differences, individual comparisons were made with the Mann–Whitney *U*-test. A *P*-value of <0.05 was accepted as significant. Regression analysis was performed by Spearman's rank correlation. Data were analysed with the MINITAB standard statistical package (Minitab Inc., State College, PA).

RESULTS

Proliferative response of spleen lymphocytes to OVA *in vitro*

The proliferative response of rat lymphocytes to OVA and BSA from sensitized animals was tested at different time-points after sensitization. Spleen lymphocytes from sensitized ($n=4$) and naive ($n=4$) animals were cultured in the presence of OVA or BSA over periods of 48, 72, 96 and 168 hr. Cell cultures showed a maximal proliferative response 96 hr after stimulation. This duration of culture was used for further studies. Rats receiving i.p. injections (days 1–3) 3 days later (day 6) had an increased level of proliferation in the presence of OVA compared with BSA ($P<0.03$). This response was further increased and reached a peak on day 7 ($P<0.03$; OVA versus BSA) (Fig. 1a.). A small increase was observed in the proliferative response to BSA in some of the samples from sensitized animals when compared to non-sensitized controls (data not shown). Therefore, the specificity of this proliferation has been tested in a dose–response study. While OVA induced proliferation of cells in a dose-dependent manner (within the dose range of 0·25–250 μg/ml), there was no such response to BSA (Fig. 1b.); furthermore, no proliferative response was observed in samples from non-sensitized animals (data not shown).

Effect of adoptive transfer of total T cells on airway responsiveness

Naive recipients were injected i.v. with a total of 50×10^6 T-lymphocyte-enriched spleen cells from either OVA- or saline-injected donors. The purity of the transferred cell population was 95·5 ± 8·1%, as assessed by flow cytometric analysis using a mouse anti-rat CD2 monoclonal antibody. A group of naive control rats was injected i.v. with saline. All recipient rats were exposed to OVA aerosol 24 hr after injection of cells or saline. After a further 24 hr, airway responsiveness to ACh was measured (Fig. 2a). Rats that received enriched spleen T cells from sensitized animals showed a leftwards shift of the dose–response curve, with increased lung resistance following increasing concentrations of inhaled ACh, significant at the doses of $10^{-3.5}$ ($P<0.05$) and $10^{-3.0}$ M ($P<0.05$) compared with recipient rats of non-sensitized cells. In addition, mean PC_{100} and PC_{200} values were significantly lower in the group that received sensitized T cells ($P<0.01$ and $P<0.05$, respectively) (Fig. 3a). $-\log_{10}$ PC_{100}, PC_{200} and PC_{300} values in the saline injected group were 2·33 ± 0·09, 2·01 ± 0·07 and 1·87 ± 0·03, respectively. Baseline resistances showed no significant differences from the control group (Table 1).

Cellular content of BAL

There was no statistically significant difference in the total number of cells ($\times10^6$) recovered from BAL fluid in sensitized and naive T-cell recipients (3·40 ± 0·34 and 3·73 ± 0·39, respectively). Saline-injected rats had a total cell count of 2·2 ± 0·2 ($\times10^6$), and a macrophage, eosinophil, neutrophil and lymphocyte count of 201·4 ± 17·6, 1·8 ± 0·4, 9·4 ± 2·7 and 11·3 ± 0·4 ($\times10^4$), respectively. There was a trend for an increase in the numbers of eosinophils and lymphocytes in the animals that had received sensitized T cells compared with recipients of naive cells, but these differences did not achieve statistical significance (Fig. 4a).

Eosinophil counts in the mucosal tissue

Recipients of sensitized T cells had significantly higher numbers of eosinophils than control recipients as assessed by counting the BMK-13$^+$ cells/mm^2 (mean ± SEM were 263·7 ± 42·5 and 58·1 ± 36·7, respectively; $P<0.02$) (Figs 4b and 5). Saline-injected rats had a tissue eosinophil count of 6·7 ± 1·3. There was a significant correlation between the number of BMK-13$^+$ (MBP$^+$) cells and the increase in lung resistance at an ACh concentration of 10^{-3} M/l (Spearman correlation coefficient $r=0.78$; $P<0.03$). In addition, the $-\log PC_{100}$ correlated with eosinophil infiltration in rats that received sensitized T cells ($r=0.75$; $P<0.03$). The control group receiving naive cells did not show such a correlation.

Effect of adoptive transfer of CD4$^+$ T cells on airway responsiveness

Naive recipients were injected i.v. with a total of either 20×10^6 ($n=8$) or 5×10^6 ($n=8$) CD4$^+$ T cells from sensitized donor rats. Control rats received 20×10^6 CD4$^+$ from saline-injected donors ($n=8$). The purity of the transferred cell population was 98·0 ± 2·8%, as assessed by FACS analysis. The contaminating cells were less than 1% CD8$^+$ (OX8$^+$) and less than 1% B cells (OX33$^+$). All recipient rats were exposed to OVA aerosol 24 hr after adoptive transfer. After a further 24 hr, airway responsiveness to ACh was measured (Fig. 2b). Rats that received 20×10^6 CD4$^+$ spleen T cells from sensitized

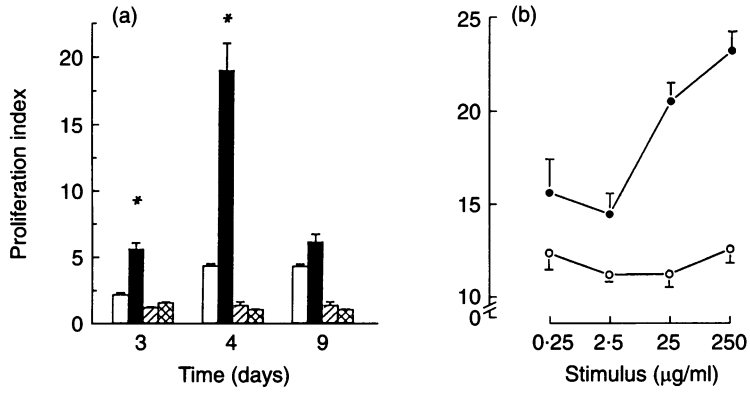

Figure 1. (a) Proliferative response of spleen lymphocytes to OVA at different time-points after i.p. sensitization. Spleen lymphocytes from sensitized and naive animals were cultured in the presence of OVA (solid bars and crossed bars, respectively) or BSA (open bars and hatched bars, respectively) for 96 hr ($n=4$). Cells from rats receiving i.p. sensitization showed an increased level of proliferation in the presence of OVA on days 6, 7 and 12 (3, 4 and 9 days after completing the 3-day sensitization course) compared with BSA controls. Results are expressed as mean ± SEM; * $P<0{\cdot}03$. (b) Proliferative response of spleen lymphocytes to different concentrations of OVA and BSA. Spleen lymphocytes from rats injected with OVA/Al(OH)$_3$ on 3 consecutive days were tested for their proliferative response to different concentrations of OVA (closed circles) or BSA (open circles) on day 7 (4 days after the last i.p. injection). Cells (2×10^6/ml) were incubated at 37C° for 96 hr and were harvested after 12 hr pulsing with [^{3}H]thymidine. Results are expressed as mean ± SEM.

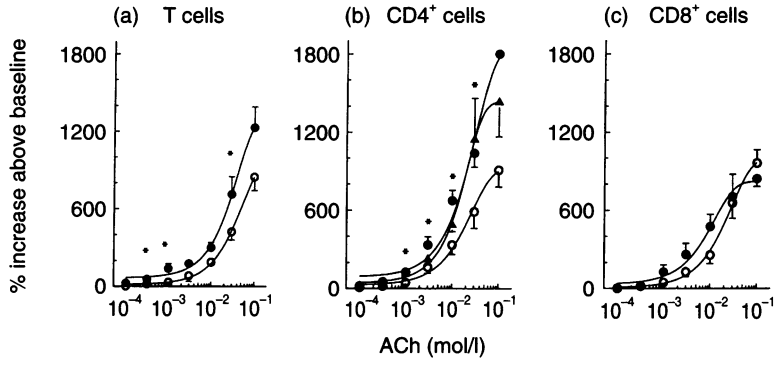

Figure 2. Effect of adoptive transfer of T cells on airway responsiveness on ACh dose–response. (a) Naive recipients were injected i.v. with a total of 50×10^6 T-lymphocyte-enriched spleen cells from either OVA-sensitized (closed circles; $n=18$) or saline-injected (open circles; $n=12$) donors. All recipients were exposed to OVA aerosol 24 hr after adoptive transfer. Airway responsiveness to ACh was measured in the animals 24 hr later. Points represent percentage increase in airway resistance above baseline. Results are expressed as mean ± SEM; *$P<0{\cdot}05$. (b) Naive recipients were injected i.v. with a total of either 20×10^6 ($n=8$; closed circles) or 5×10^6 ($n=8$; closed triangles) CD4$^+$ T cells from sensitized donor rats. Control rats received 20×10^6 CD4$^+$ cells from saline-injected donors ($n=8$; open circles). All recipient rats were exposed and measured as above. Points represent percentage increase in airway resistance above baseline. Results are expressed as mean ± SEM; *$P<0{\cdot}05$. (c) Naive recipients were injected i.v. with a total of 5×10^6 ($n=8$; closed circles) CD8$^+$ T cells from sensitized donor rats. Control rats received 5×10^6 CD4$^+$ cells from saline-injected donors ($n=8$; open circles). All recipient rats were exposed and measured as above. Points represent percentage increase in airway resistance above baseline. Results are expressed as mean ± SEM; *$P<0{\cdot}05$;.

animals showed a leftwards shift of the dose–response curve, with increased lung resistance following increasing concentrations of inhaled ACh, significant at doses of $10^{-3{\cdot}0}$ ($P<0{\cdot}02$), $10^{-2{\cdot}5}$ ($P<0{\cdot}03$) $10^{-2{\cdot}0}$ ($P<0{\cdot}02$) and $10^{-1{\cdot}5}$ M ($P<0{\cdot}05$) compared with recipient rats of 20×10^6 non-sensitized CD4$^+$ T cells. Mean PC_{100}, and PC_{200} values were significantly lower in the group that received sensitized T cells ($P<0{\cdot}01$ and $P<0{\cdot}05$, respectively) (Fig. 3b). Baseline resistances (Table 1) were not different between these two groups of rats. Although injection of sensitized CD4$^+$ T cells appeared to induce dose-related changes in airway responsiveness, recipient rats of 5×10^6 sensitized CD4$^+$ cells did not show significant differences from recipients of naive cells.

Cellular content of BAL and tissue eosinophil numbers in recipient rats of CD4$^+$ cells

There was no statistically significant difference in the total number of cells recovered from BAL fluid in sensitized (5×10^6 and 20×10^6) and naive (20×10^6) CD4$^+$ T-cell recipients ($3{\cdot}01\pm0{\cdot}49$, $5{\cdot}55\pm0{\cdot}69$ and $3{\cdot}64\pm0{\cdot}56\times10^6$, respectively). There was a trend for an increase in the numbers of eosinophils, neutrophils and lymphocytes in the animals that had received sensitized T cells, but these differences did not attain statistical significance compared with recipient rats of non-sensitized cells (Fig. 6a). Eosinophils (BMK-13$^+$ cells), however, were present in a significantly elevated number in the airway mucosal tissue of recipients of sensitized CD4$^+$ cells ($P<0{\cdot}05$) (Fig. 6b.)

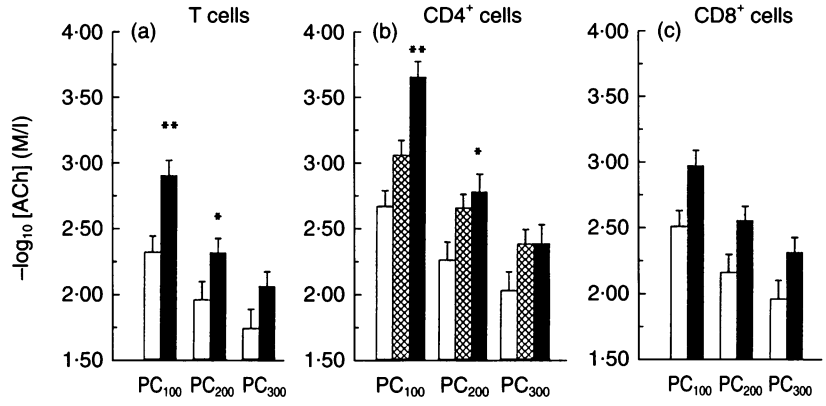

Figure 3. Effect of adoptive transfer of T cells on PC_{100}, PC_{200} and PC_{300}. The concentrations of ACh that caused 100%, 200% or 300% increases above baseline lung resistance were calculated by extrapolation of individual dose–response curves. The mean dose–response curves are shown in Fig. 2. Experiments and measurements were performed as explained in Fig. 2. Data are expressed as mean ± SEM; $*P<0.05$ $**P<0.01$. Open bars: recipients of naive cells. Hatched bars: recipients of 5×10^6 $CD4^+$ sensitized T cells (middle panel only). Solid bars: recipients of sensitized cells.

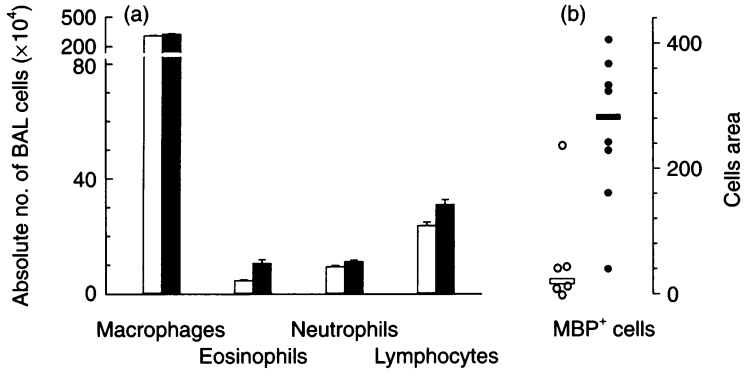

Figure 4. (a) Cellular content of BAL fluid after total T-cell transfer. BAL was performed using a total volume of 20 ml of saline in 2-ml fractions followed by Giemsa staining of cytospin preparations. Differential cell count was made by counting one thousand cells under high power (×500). The absolute cell count was calculated by multiplying differential cell counts by the total cell number recovered from BAL of individual samples. Solid bars: recipients of sensitized cells. Open bars: recipients of naive cells. Results are expressed as mean ± SEM. (b) Effect of adoptive transfer of total T cells on number of MBP^+ cells in the mucosal tissue of recipient rats. Immunocytochemistry was performed as explained in Fig. 5. Solid circles: recipients of sensitized cells. Open circles: recipients of naive cells. Horizontal bars represent the median values of positive cell numbers; Comparison using non-parametric Mann–Whitney test showed a significant difference between the two groups ($P<0.002$).

Effect of adoptive transfer of $CD8^+$ T cells on airway responsiveness, cellular content of BAL and tissue eosinophil numbers

Naive recipients were injected i.v. with a total of 5×10^6 $CD8^+$ spleen cells from either OVA- or saline-treated donors. The purity of the transferred cell population was 95·1 ± 6·9%, as assessed by flow cytometric analysis using a mouse anti-rat CD8 monoclonal antibody (OX8). All recipient rats were exposed to OVA aerosol 24 hr after injection of cells or saline. After a further 24 hr, airway responsiveness to ACh was measured (Fig. 2c). Rats that received sensitized $CD8^+$ T cells did not show significant differences in their dose–response curves to ACh compared with recipient rats of non-sensitized cells. There was no significant difference in their PC_{100}, PC_{200} or PC_{300} values (Fig. 3c) or in their baseline resistances (Table 1).

There was no statistically significant difference in the total number of cells or in the numbers of eosinophils, neutrophils and lymphocytes recovered from BAL fluid in sensitized and naive $CD8^+$ T-cell recipients (Fig. 7a), or in the numbers of eosinophils in the airway mucosal tissue of these rats (Fig. 7b).

DISCUSSION

Data from previous studies suggested that i.p. sensitization and inhalational exposure to OVA induced pathological changes in BN rats reminiscent of human atopic asthma. It has been speculated that OVA-specific memory T cells that home to airway tissue become activated following antigen presentation, and produce cytokines that favour the development of an eosinophilic inflammation. Eosinophils attracted to the airway tissue in turn may damage the epithelium and produce hyperresponsiveness. If T lymphocytes had a major function in this process, transfer of these cells from sensitized to naive animals should alone result in eosinophilia and AHR after OVA exposure.

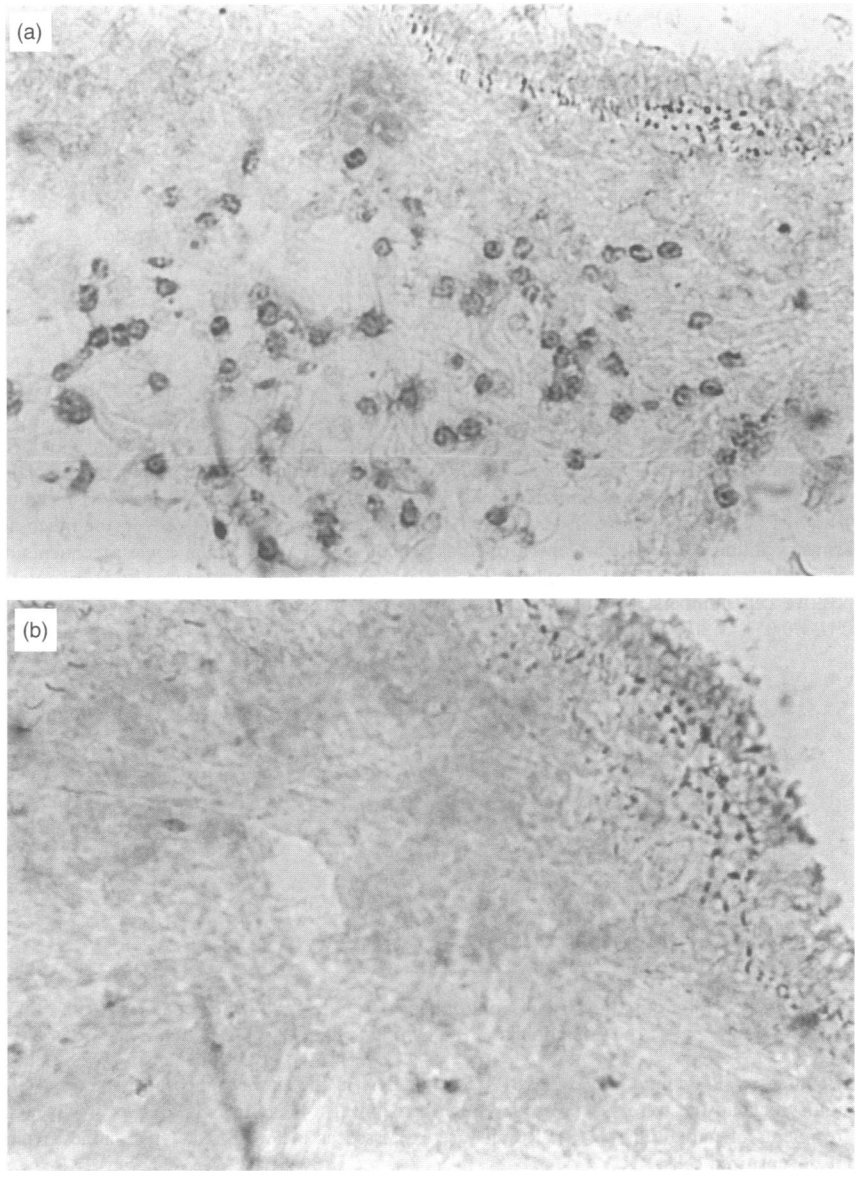

Figure 5. Bronchial tissue labelled with BMK-13 monoclonal antibodies. Immunohistology staining for MBP was performed on randomly selected subsets of the group that received sensitized cells ($n=8$) and recipients of naive cells ($n=6$). Cryostat sections (6 μm) of bronchial and lung tissue were incubated with mouse monoclonal antibody BMK-13. After labelling with the second layer (rabbit anti-mouse IgG), positive staining was visualized by the alkaline phosphatase–anti-alkaline phosphatase technique. Specifically bound alkaline phosphatase was detected as a red colour. Slides were read in a blind fashion and in coded random order by two observers. Counts were expressed as positive cells per mm^2 of cross-sectional subepithelial surface. (a) A representative section of recipient rat of T cells from a sensitized donor. (b) Recipient rat of T cells from a non-sensitized donor ($\times 300$).

We have shown in this study that transfer of both total and CD4$^+$ T cells obtained from spleens of rats sensitized to OVA induced airway hyperresponsiveness in naive recipients, and was associated with a significant airway wall infiltration of eosinophils. These results strongly support the view that activated T cells, which have been observed previously in BAL[14] and airway mucosal tissue[15] of sensitized and challenged BN rats, may be involved primarily in both the recruitment of eosinophils and the AHR following allergen exposure.

Initial questions of this study related to whether the cell population to be transferred contained antigen-specific cells. Spleen T cells have been shown previously to transfer effectively the immunological responsiveness to naive recipients shortly after sensitization of donor animals.[21] The time interval we used for the transfer of spleen cells from OVA-sensitized rats into recipients was based on *in vitro* studies. The maximum proliferative response of spleen cells from sensitized rats to OVA was found to occur 4 days after the last dose of i.p. OVA/Al(OH)$_3$ (day 7). Proliferation of cells from sensitized rats was dependent on the dose of OVA and no response was observed in cell cultures from naive animals or in those with BSA, indicating that spleen cells transferred in that day are at least partly reactive to OVA.

The regulation of T-cell migration in the airway tissue is obscure. Lymphocytes labelled *in vitro* and injected i.v. in different species migrate rapidly (within 15 min) to the lung

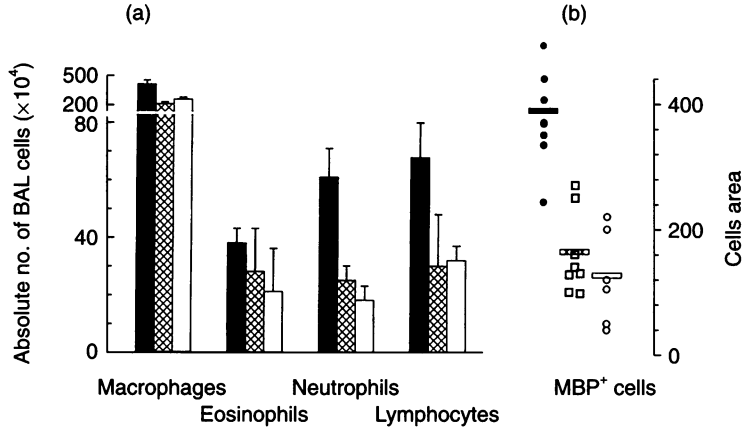

Figure 6. (a) Cellular content of BAL fluid after $CD4^+$ T-cell transfer. BAL cytospins were prepared as explained in Fig. 5. Open bars: recipients of naive cells ($n=7$). Hatched bars: recipients of 5×10^6 $CD4^+$ sensitized T cells ($n=8$). Solid bars: recipients of sensitized cells ($n=8$). (b) Effect of adoptive transfer of total T cells on number of MBP^+ cells in the mucosal tissue of recipient rats. Immunocytochemistry staining for MBP was performed as explained in Fig. 5b. Open circles: recipients of naive cells. Open squares: recipients of 5×10^6 sensitized $CD4^+$ T cells. Solid circles: recipients of sensitized cells; Horizontal bars represent the median values of positive cell numbers. Comparison between the rats receiving 20×10^6 sensitized and naive cells showed a significant difference ($P<0.05$).

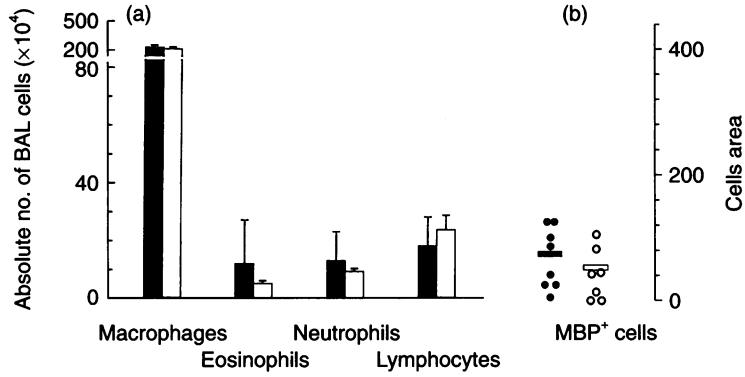

Figure 7. (a) Cellular content of BAL fluid and (b) MBP^+ cell numbers in the airway mucosal tissue after $CD8^+$ T-cell transfer. Cytospins and immunocytochemistry were performed as described in Figs 4a and 5. Solid bars: recipients of sensitized cells ($n=8$). Open bars: recipients of naive cells ($n=8$). Results are expressed as mean $\pm$ SEM. Open circles: recipient of naive cells. Solid circles: recipient of sensitized cells.

and persist there for 48 hr.[29] In the present study, allergen exposure was performed 24 hr after i.v. injection of cells. This time-point was based on the above observation and that of Garssen *et al.*,[23–25] in which they achieved successful transfer of AHR using a similar time-point for challenge. After aerosol exposure to OVA, allergen-specific memory cells, having settled in the mucosal tissue, may become activated following antigen presentation by macrophages and dendritic cells. These are abundant in the airway mucosal tissue and have been shown to be the most important antigen-presenting cells for inhaled antigens.[30] It should be noted that the effect of a single allergen exposure alone (without cell transfer or presensitization of the rats) is not sufficient to increase airway responsiveness or induce eosinophilia.[31]

Activated T cells have the potential to synthesize and release proinflammatory cytokines. They are thus capable of initiating a cascade of events, which include promoting further migration of immunocompetent and inflammatory cells into sites of antigen presentation, resulting in increased airway responsiveness. In the present study, an increase in airway responsiveness was observed 24 hr after OVA challenge of animals that received sensitized spleen T cells, compared with recipient rats of non-sensitized cells.

The exact mechanisms by which T cells may affect airway responsiveness are poorly understood. Interaction between activated T cells and eosinophils may be of major importance.[5–7,10–12,32–36] Proinflammatory cytokines produced by T lymphocytes are involved in eosinophil maturation, migration, prolonged survival and activation.[37–40] Activated eosinophils release a variety of cationic proteins, including MBP, eosinophil cationic protein and eosinophil peroxidase, with the potential to injure the respiratory epithelium resulting in development of increased sensitivity of the airways to non-specific bronchoconstrictor agents. Studies in primates and in rats have shown that administration of human MBP produces airway constriction and AHR.[41,42] Significant correlations have also been shown between MBP^+ cells and AHR in human asthma.[5] The present findings of increased expression of MBP immunoreactivity in the airway mucosal tissue contribute to these observations. The correlation between the number

of MBP$^+$ cells and the increase in airway resistance to ACh supports the hypothesis that T cells exert their regulatory role through the initiation of an eosinophilic inflammation in this BN rat model.

In previous studies, transfer of non-purified lymphoid cell populations[43,44] from peribronchial lymph nodes and spleens of OVA- and ragweed-sensitized BALB/c mice led to increases in the responsiveness of tracheal smooth muscle to electrical field stimulation *ex vivo*, elevation of allergen-specific IgE and specific immediate hypersensitivity responses. This model suggested a causal relationship between IgE-mediated mechanisms and airway hyperresponsiveness with no independent role established for T cells.

In another animal model, purified populations of T cells were transferred from picryl chloride-sensitized donor BALB/c mice. The recipients, after a subsequent inhalational challenge, had a heightened tracheal reactivity to carbachol *in vitro*.[23–25] While these experiments proposed a role for T cells in initiating AHR directly, the picryl-chloride model is based on a delayed-type hypersensitivity reaction, with no evidence of eosinophilic airway inflammation or IgE production, thus having little in common with human asthma. In this regard, the present study provides an important model of allergic sensitization and eosinophilic airway inflammation, where AHR and airway eosinophilia are shown to be transferred *in vivo* by i.v. injection of T cells.

CD4$^+$ lymphocytes may bear special importance in the development of allergic inflammation since inert, small protein molecules, such as allergens, are processed by antigen-presenting cells and presented via major histocompatility complex (MHC) class II to CD4$^+$ T cells (as opposed to viral antigens, which are presented by all body cell types via MHC class I to CD8$^+$ T cells). In addition, these cells are capable of producing IL-4 and providing adequate cell–cell contact for B lymphocytes for IgE production, and further, releasing IL-5, the exclusive cytokine that promotes eosinophil maturation from its precursors. In fact, one of the first observations to focus attention on the T cell in asthma pathogenesis derived from studies on patients with acute severe asthma showing increases in CD25 expression associated with CD4$^+$ rather than CD8$^+$ T-cell populations.[12] In addition, in BAL samples of asthmatic patients, CD4$^+$ but not CD8$^+$ T cells were activated in association with production of mRNA for Th2-type cytokines (IL-4 and IL-5),[45] and in mild asthmatics that showed both CD4$^+$ and CD8$^+$ T-cell activation in BAL fluid, only the numbers of activated CD4$^+$ cells correlated with eosinophilia and disease severity.[32] Finally, in animal models it was found that depletion of CD4$^+$ cells inhibited both airway inflammation and AHR.[46–48] These data suggest that CD4$^+$ T cells have a major importance in regulating events leading to allergic airway inflammation, and CD8$^+$ cells may bear little if any significance.

Nevertheless, recent studies showed that CD8$^+$ T lymphocytes, which used to be regarded simply as cytotoxic cells, may be able to regulate immune responses by determining the pattern of cytokines produced by CD4$^+$ T cells and the isotype of immunoglobulins expressed by B cells. Although they suppress IgE production,[21] they may still be implicated in recruitment of eosinophils through production of IL-5[49] and lymphocyte-chemotactic factor produced by histamine-stimulated CD8$^+$ T cells.[50] There is also evidence of functionally distinct subsets of CD8$^+$ T cells capable of synthesizing different combinations of cytokines.[51–53]It is possible that CD8$^+$ T cells have an immunoregulatory role since they appear to be active early in the immune reaction.[21] Indeed, in our previous studies we found a significant increase in the number of CD8$^+$ T cells in the airway mucosal tissue by immunocytochemistry 24 hr after OVA challenge of sensitized BN rats, while CD4$^+$ lymphocyte numbers remained unchanged.[15]

Taken together, both subsets of T cells are potentially capable of inducing such changes. Therefore, in the present study we purified T cells by magnetic beads to CD4$^+$ and CD8$^+$ populations and injected them i.v. into different groups of naive recipient rats. Using the same protocol as for total T-cell transfer, it was demonstrated that CD4$^+$ T cells but not CD8$^+$ T cells, when injected i.v., are capable of inducing allergic AHR directly following a single challenge of the recipient rats. These results strengthen an independent role of CD4$^+$ T cells in allergic AHR and form an important step in further elucidating asthma pathogenesis.

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